Journal of Environmental Management 228 (2018) 451–457

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Glyphosate and aminomethylphosphonic acid degradation in biomixtures based on alfalfa straw, wheat stubble and river waste

M.R. Lescano[a], L. Pizzul[b], M.d.P. Castillo[b], C.S. Zalazar[a,c,*]

[a] Instituto de Desarrollo Tecnológico para la Industria Química (INTEC, UNL-CONICET), 3000, Santa Fe, Argentina
[b] RISE- Research Institutes of Sweden, Uppsala, S-750 07, Sweden
[c] Dep. Medioambiente, FICH-UNL, Ciudad Universitaria, 3000, Santa Fe, Argentina

ARTICLE INFO

Keywords:
Biopurification systems
Biobeds
Herbicide
Wastewater
AMPA

ABSTRACT

The aim of the work was to evaluate novel biomixtures for their use on biopurification systems (BPS) in Argentina also called biobeds. Glyphosate and aminomethylphosphonic acid (AMPA) degradation was evaluated on biomixtures containing local materials: alfalfa straw (As), wheat stubble (Ws), river waste (Rw) and soil. Glyphosate, AMPA concentrations and biological activity were followed with time. Soil was used as control. Glyphosate initial concentration was 1000 mg kg^{-1}. Glyphosate disappeared almost completely after 63 days in all tested biomixtures. For Ws, WsRw and AsRw glyphosate degradation was around 99% and for As 85%. The biomixture Ws showed the highest glyphosate degradation rate. In all cases AMPA was formed and degraded to concentrations between 60 and 100 mg kg^{-1}. In the control with only soil, glyphosate was degraded 53% and AMPA concentration at the end of the test was 438 mg kg^{-1}. We conclude that alfalfa straw, wheat stubble and river waste are local materials that can be used in the preparation of biomixtures since they showed higher glyphosate degradation capacity and less AMPA accumulation compared to the soil alone. Also, the presence of river waste did enhance the water retention capacity.

1. Introduction

Environmental contamination by pesticides may be caused from point or diffuse sources. Diffuse contamination takes place during the application of pesticides in the field, mainly due to runoff or drift losses. Point source contamination occurs as a result of accidental spills at the place of pesticide manipulation, during the filling of the spraying equipment or due to the management of pesticides residues left outside and inside the tank. Even when precautions are taken, there is a risk of potential ground and surface water contamination (Castillo et al., 2008).

Biopurification systems (BPS), as biobeds and biofilters, are designed to collect and decontaminate accidental spills or waste liquids with a high concentration of pesticides and, therefore, avoid the contamination of surface- and ground waters. BPS are low cost systems that basically consist of waterproofed excavations or containers filled with a biologically active matrix, called biomixture, and covered by a vegetal layer (Castillo et al., 2008). The biomixture consists on soil, lignocellulosic materials and a humified organic substrate mixed at variable volumetric ratios. It has a high microbial activity and it is the main component of the BPS, allowing the retention and subsequent biological degradation of the pesticides. The original biomixture that was designed for the Swedish biobed (Torstensson and Castillo, 1997) was made of wheat straw (rich in lignocellulose), soil and peat. The straw allows the development of ligninolytic fungi that in turn promote the enzymatic degradation of pesticides. The soil provides adsorption capacity and acts as a source of pesticide-degrading bacteria. Peat also contributes to pesticide adsorption, moisture control and the decrease of pH that promotes the growth of fungi (Castillo and Torstensson, 2007).

In order to achieve a low cost and sustainable BPS, the composition of the biomixture must be adapted according to the availability of agricultural residues and local materials. For example, straw has been replaced by sunflower, olive and vine crop residues (Karanasios et al., 2010), bagasse (Roffignac et al., 2008) or oat husks, barley husks or sawdust (Urrutia et al., 2013). Furthermore, an important challenge is to find materials that can replace peat as it is a scarce and expensive resource (Gao et al., 2015; Karas et al., 2015). Peat has been replaced by different types of compost (Omirou et al., 2012; Karanasios et al., 2010) or spent mushroom substrate (Gao et al., 2015).

In order to use the BPS in Argentina it is necessary to find biomixtures based on locally available materials but that can still keep high degradation efficiencies. Once the crops are harvested, a significant amount of "crop residues" are left and they are commonly called stubbles. The most available lignocellulosic materials in Argentina are wheat stubble and

* Corresponding author. Instituto de Desarrollo Tecnológico para la Industria Química (INTEC, UNL-CONICET), 3000, Santa Fe, Argentina.
E-mail address: szalazar@santafe-conicet.gov.ar (C.S. Zalazar).

https://doi.org/10.1016/j.jenvman.2018.09.009
Received 20 March 2018; Received in revised form 24 August 2018; Accepted 2 September 2018

alfalfa straw. In 2016, the total cereal production in Argentina was 67 million tons (of which about 80% corresponds to corn and wheat) and the alfalfa forage and silage production reached 39 million tons according to FAO 2016 (FAOSTAT, 2016). An alternative material to peat is river waste that consists on plant residues accumulation under anaerobic conditions, common in certain areas of the Paraná Delta River (Di Benedetto et al., 2004) and that is currently used in flower production for the formulation of substrates.

It has been demonstrated that several enzymes can act in pesticides degradation in soil, such as phosphatases, hydrolases and carboxylesterases (Tortella et al., 2012). In this sense the determination of biological activities such as hydrolytic activity based on the Fluorescein Diacetate activity (FDA) is one approach to monitor the potential effect of pesticides in biological activities. FDA (3′,6′-diacetylfluorescein) has been used to determine amounts of active fungi and bacteria since it is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases (Schnürer and Rosswall, 1982).

Glyphosate [N-(phosphonomethyl) glycine], a synthetic phosphonate compound with stable carbon-phosphorus (C—P) bond is the active ingredient of broad spectrum post-emergent, and non-selective systemic herbicide (Li et al., 2016). Glyphosate-based formulations have become the dominant herbicides on a global scale (Cuhra et al., 2016). Recent studies revealed that glyphosate occurs in soil, surface water, and groundwater, and residues are found at all levels of the food chain, such as drinking water, plants, animals, and even in humans (Milan et al., 2018). Aminomethylphosphonic acid (AMPA) is a degradation product resulting from phosphonate degradation (Wang et al., 2016). It can be a metabolite of glyphosate microbial degradation in soils (Borggaard and Gimsing, 2008). In a spatially wide occurrence study, Battaglin et al. (2014) showed that glyphosate is detected without AMPA in only 2.3% of 3732 water and sediment samplesand that AMPA is detected without glyphosate in 17.9% of samples. AMPA is strongly adsorbed to soil particles and moves with the particles towards the stream in rainfall runoff. In urban areas, AMPA comes from phosphonates and glyphosate in wastewater. AMPA is reported to be persistent in soils and sediments (Grandcoin et al., 2017). Based on recent reports on potential chronic side effects of glyphosate (Battaglin et al., 2014), the World Health Organization reclassified the herbicide glyphosate as probably carcinogenic to humans in 2015 (WHO, 2015).

In Argentina the use of glyphosate pesticide increased from 1 million liters in 1991 to 200 million liters in 2013 (Casafe, 2013; Binimelis et al., 2009). The occurrence of glyphosate and AMPA have been reported in the water and sediments of streams from rural and suburban basins of our country (Argentina) within the provinces of Buenos Aires, Santa Fe and Córdoba (Castro Berman et al., 2018).

In this context, the aim of this work was to evaluate glyphosate and AMPA degradation employing different biomixtures prepared with local materials (soil, alfalfa straw, wheat stubble and river waste).

Glyphosate degradation and the presence of its main metabolite AMPA were followed with time. Biological activity, as fluorescein diacetate hydrolysis (FDA), was also followed. Soil alone was run as control.

2. Materials and methods

2.1. Biomixtures preparation

Biomixtures were prepared using an agricultural soil, two different lignocellulosic materials (alfalfa straw or wheat stubble) and river waste. Experiments were conducted using three independent replicates. The soil was obtained from a field in the north of Santa Fe province, Argentina (29° 42′ 59″ S and 60° 5′ 35″ W) with more than 20 years of continuous soybean cultivation where glyphosate was applied. The local soil acts as an inoculum of pesticide-adapted microbiological populations (De Wilde et al., 2007). The kind of soil according to its taxonomy is aquic Argiudoll. Alfalfa straw and wheat stubble were collected from the same field as the soil. River waste is a commercial product (Santa Isabel S.A. vivarium), used for cultivation of plants. Physicochemical properties of all components are shown in Tables 1–3.

Table 1
Soil physicochemical properties.

Parameter	Soil
Granulometry (%)	Sand 6.4; Silt 66.6; Clay 27.0
C (g kg^{-1})	19.7
Organic matter ((g kg^{-1}))	34.0
P (mg L^{-1})	0.023
Actual density (g cm^{-3})	2.67
Porosity (%)	70.7
pH[a]	5.96
Ashes ((mg kg^{-1}))	948.
K[b] (mg kg^{-1})	462.7
Ca[b] (mg kg^{-1})	184.9
Mg[b] (mg kg^{-1})	84.4
Na[b] (mg kg^{-1})	10.4
N (g kg^{-1})	1.53
C/N Ratio	12.9

[a] Determined in a mixture of air-dried soil and deionized water (1:2.5 w/v).
[b] Values corresponding to total content.

Both glyphosate and AMPA concentrations were below the Limit of Detection in all substrates (0.6 μg L^{-1} for both according the method reported by Sasal et al., 2015). The soil was sieved (3 mm), the stubble and straw were cut into pieces of approximately 2–3 cm and the river waste was used directly.

The biomixtures were prepared by mixing the components with a shovel in the proportions shown in Table 4 and 15 L of biomixture were placed in 30 L glass boxes (20 cm × 30 cm × 50 cm) (Fig. 1). Temperature, pH and moisture (expressed as water weight/dry material weight) were registered daily. Moisture was adjusted to 60–70% and kept constant during the whole experiment by adding distilled water. Moisture and pH measurements were performed with a garden meter (TFA). Soil as the only component was run in parallel as control. The moisture was adjusted at the same value range as recommended by Castillo et al. (2008).

After preparation the biomixtures were maturated for 50 days before the addition of glyphosate according to the recommendation of previous studies (Castillo et al., 2008; Roffignac et al., 2008; Karanasios et al., 2012; Góngora-Echeverría et al., 2017). The commercial glyphosate formulation Eskoba® was sprayed over the surface of the biomixtures and soil alone at a concentration of 1000 mg glyphosate kg^{-1} dry biomixture. This high concentration value selected is related to the residues produced in the area (mainly rinsing water of commercial containers and water belonging from spray tank washing (De Wilde et al., 2007).

The experience was performed for 63 days and samples were taken immediately after glyphosate application (day 0) and after 10, 16, 25, 43 and 63 days. The experience time up to 63 days was chosen taking into account the half-life of glyphosate in soil and according previous studies of glyphosate degradation in biomixtures (Roffignac et al., 2008; Góngora-Echeverría et al., 2017); a typical field half-life of 47 days has been suggested (Lawrence, 2002).

Each sample was a composite of several subsamples taken at different positions of the biobed employing a soil sampler and was

Table 2
Physicochemical properties of the lignocellulosic materials.

Parameter	Alfalfa straw	Wheat stubble
Organic matter (%)	79.5	82.2
Dry material (%)	89.6	91.3
Ashes (%)	10.1	9.1
Raw or crude fiber (%)	23.6	38.4
P (%)	0.4	Not detected
N (%)	2.3	0.46
Density (g cm^{-3})	0.08	0.06

Table 3
Physicochemical properties of the river waste.

Parameter	River waste
Organic matter (%)	18.2
C dry base (%)	10.6
pH (1:2,5)	4.2
Actual density dry base (g cm^{-3})	1.83
Apparent density dry base (g cm^{-3})	0.39
Moisture (%)	19.7
Ashes (%)	71.9
P dry base (mg L^{-1})	8.47
N (%)	0.57
C/N Ratio	18.6

Table 4
Biomixtures composition.

	Soil (%)	Alfalfa Straw (As) (%)	Wheat stubble (Ws) (%)	River waste (Rw) (%)
As	50	50	–	–
AsRw	25	50	–	25
Ws	50	–	50	–
WsRw	25	–	50	25

Fig. 1. Biobeds at laboratory scale.

analyzed in triplicate. At each sampling occasion the concentration of glyphosate and AMPA was determined and the evolution of biological activity as a function of time was followed. In addition the community of yeast, fungi and total viable mesophilic bacteria was estimated at the beginning and at end of the experiment. When necessary, moisture content was restored spraying distilled water on the biomixtures.

2.2. Chemicals

The following chemicals were used: commercial glyphosate formulation, N-phosphonomethylglycine salt 35.6% as acid, active compound (Eskoba®, Red Surcos, Argentina); p-toluensulphonil chloride (Sigma Aldrich); AMPA and glyphosate standards (Sigma Aldrich); fluorescein diacetate (Sigma, Sigma Aldrich), fluorescein sodium salt (Fluka, Sigma Aldrich) and acetone (Merck). All other chemicals were purchased from Cicarelli. For estimation of bacteria and yeast and fungi community, Nutritive Agar (NA, Britania) and Fungi and Yeast (H y L, Britania) medium culture were used and meat peptone (Merck) was used for all dilutions.

2.3. Glyphosate and AMPA analysis

Glyphosate and AMPA extraction was carried out as follows: 20 mL of 0.1 M KH_2PO_4 was added to 10 g of air dried and milled sample in a 50 mL centrifuge tube and vigorously mixed by hand and shaken at 250 rpm for 1 h in an orbital shaker. Afterwards the sample was sonicated for 1 h and centrifuged for 40 min at 2190*g* and the supernatant was filtered through 0.45 µm nylon syringe filters. The derivatization procedure, based on Kawai and Uno (1991), with slight modifications, was carried out employing p-toluene sulphonyl chloride (TsCl): 500 µL of phosphate buffer (pH 11) and 200 µL of the derivatizing agent were added to 1 mL of sample and then heated to 50 °C for 5 min. The obtained sample was analyzed by HPLC/UV Waters® equipped with a C-18 column (X-Terra® RP). Phosphate buffer (NaH_2PO_4 0.2 M; pH = 2.3) – acetonitrile (85:15 v/v) was used as the mobile phase. The analysis was performed at a wavelength of 240 nm. A calibration curve was performed, being the linear range 10–80 mg L^{-1}, and LOD = 10 mg L^{-1} (AMPA and glyphosate) AMPA and glyphosate recoveries values ranged from 70 to 80%.

2.4. Determination of biological activity

Total (hydrolytic) microbial activity in all degradation assays was measured through the fluorescein diacetate hydrolysis (FDA). FDA method was performed according to Schnürer and Rosswall (1982), including some adaptations. The method consists in adding 6 mL of a 60 mM potassium phosphate buffer solution (pH = 7.6) in the presence of FDA to 1 g of an air dried biomixture placed in 10 mL tubes. A blank was made with each biomixture without FDA substrate. The samples were shaken and incubated at 25 °C for 60 min. After the incubation, 6 mL of acetone was added to all tubes to stop the reaction. Subsequently, the samples were centrifuged at 986*g* for 15 min. The supernatant was filtered through 0.45 Nylon filters. The absorbance was measured at 490 nm in a Perkin Elmer® spectrophotometer. The fluorescein concentration released was calculated as reference to the calibration curve performed with standard fluorescein solutions. The results were expressed as µg of fluorescein released per gram soil and time (µg g^{-1} h^{-1}).

2.5. Estimation of microbial community

For the estimation of microbial community, 10 g of fresh sample was used. It was suspended in peptone (0.1%) and serial dilutions (in duplicate) were made when necessary for bacteria and fungi estimation. NA was used as culture media for bacteria and HyL for yeast and fungi. The samples were analyzed in duplicate. Petri dishes were incubated at 30 °C for 24 h and 7 days for bacteria/yeast and fungi community count respectively. The plate count method was used and the UFC g^{-1} were estimated (Bórtoli et al., 2012; Ratcliff et al., 2006).

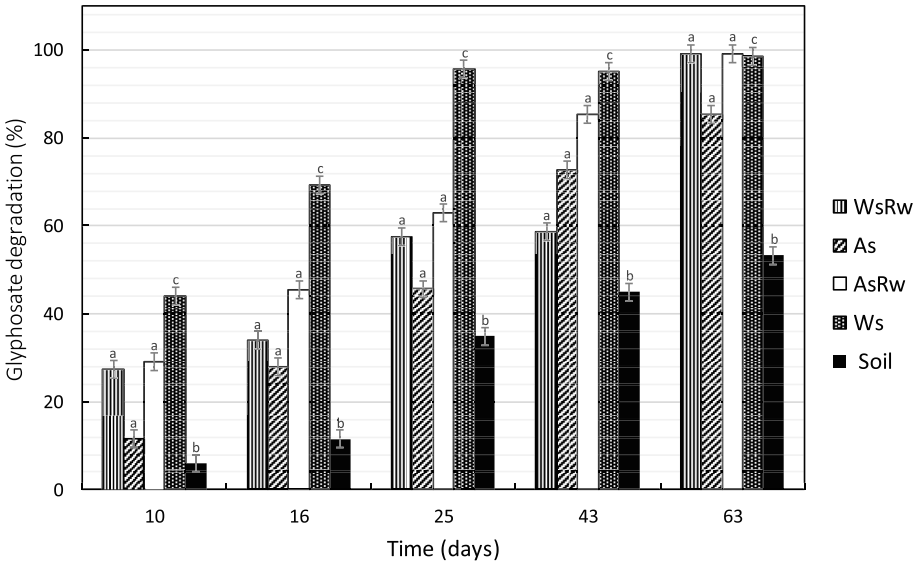

Fig. 2. Glyphosate degradation (%) in different biomixtures and soil throughout the experience. Each value is the mean of three replicates, and the errors bars show the standard deviation of the mean. Different letters refer to significant differences between glyphosate degradation means (%) taking into account the factor "biomixtures" with Duncan test ($p < 0.05$).

2.6. Statistical analysis data

Experiments were conducted using three independent replicates. Data were subjected to analysis of variance (one-way ANOVA and multifactorial ANOVA) and the averages were compared by Duncan multiple range test at 95% confidence level.

3. Results and discussion

3.1. Degradation studies

Fig. 2 shows that at 16 days of the experiment, the biomixture Ws reached 70% of glyphosate degradation. At 43 days, all biomixtures evaluated increased up to values of 60% for glyphosate degradation. In addition, glyphosate disappeared almost completely after 63 days in all tested biomixtures with the exception of As (approximately 85%), being Ws, the one which faster degradation rate. Biomixtures WsRw and AsRw also show high degradation rate. In the control with only soil, the degradation rate was lower and glyphosate was degraded 53% after 63 days. In order to reinforce the obtained results, the effect of days and biomixtures on glyphosate degradation was checked through factorial ANOVA test. Taking "days" and "biomixtures" as factors, since P-values obtained for biomixtures and days were less than 0.05 both factors have a statistically significant effect on the response "glyphosate degradation" at the 95.0% confidence level. Then the averages for the factor "biomixtures" and "days" were compared by Duncan multiple range test at the 95.0% confidence level.

The results showed that for the factor "days" there are five homogenous groups statically different. All averages for "days" were different. On the other hand it can be seen that for the factor "biomixtures" there are three homogenous groups statically different (S), (As-WsRw-AsRw) and (Ws). According to this results glyphosate degradation was significantly higher in Ws when compared with S, As, WsRw and AsRw. In addition, glyphosate degradation was significantly lower in S when compared with As, WsRw, AsRw and Ws. Biomixtures As, WsRw, AsRw has no statically differences but they had glyphosate degradation values between S and Ws.

Glyphosate half-life time (DT_{50}) was estimated by applying a logarithmic regression among glyphosate concentration vs. time. DT_{50} of 7.5, 13, 12 and 22 days were observed for Ws, WsRw, AsRw and As respectively being As the one with the highest half time (Table 5). In a biomixture the lignocellulosic material promotes the pesticide-degrading activity of lignin-degrading fungi. The wheat stubble has more raw fiber than alfalfa straw, see Table 2. Raw fiber level is related to cellulose and lignin content; therefore, the biomixtures with wheat stubble could have more activity from lignin degrading fungi (such as white rot fungi), which produce phenoloxidases (peroxidases and laccases). The broad specificity of these enzymes makes them suitable for degradation of mixtures of different pesticides (Castillo et al., 2008; Karanasios et al., 2012). On the other hand, the lower half-life corresponding to AsRw in comparison with As could be due to the different microorganism species present in the river waste.

Table 5
Glyphosate half-life time (DT_{50}) and AMPA concentration (mg kg $^{-1}$) after 63 days after glyphosate addition.

Substrate	Glyphosate DT_{50} (days)	Residual AMPA concentration (mg kg $^{-1}$)
Ws	7.5	99 ± 10
WsRw	13	97 ± 11
As	22	66 ± 10
AsRw	12	68 ± 10
Soil	32	438 ± 15

These results are similar to those found in the few published works that reported degradation of glyphosate in biomixtures. Góngora-Echeverría et al. (2017), tested the degradation of five pesticides (2,4-D, atrazine, carbofuran, diazinon and glyphosate) in biobeds systems using agricultural soil and substrates from southeastern Mexico. The biomixtures were efficient in degradation (> 99%) the pesticides in a short time, particularly glyphosate (initial concentration 360 mg L^{-1}), which was almost completely dissipated after 20 days. Roffignac et al. (2008), verified the degradation of glyphosate, malathion and lambda-cyhalothrin in a mixture of soil and bagasse. In their work, an initial glyphosate concentration of 295 mg kg^{-1} was used and 99% degradation was reached after 3 months, with a DT_{50} value of 33 days.

A wide variety of soil microorganisms, including bacteria, actinomycetes, fungi and unidentified microorganisms, can degrade glyphosate (Borggaard and Gimsing, 2008). Glyphosate can be degraded through two main pathways: one that leads to the intermediate formation of sarcosine and glycine, and the other that leads to the formation of AMPA (Dick and Quinn, 1995; Borggaard and Gimsing, 2008). AMPA is cleaved to produce inorganic phosphate and methylamine, which is ultimately mineralized to CO_2 and NH_3. AMPA is often detected in soils that have received glyphosate. On the other hand, sarcosine, has not been detected in soil, which has been attributed to its fast degradation (Borggaard and Gimsing, 2008). In the biomixtures the growth of white rot fungi is favored by the addition of materials rich in lignin. The production of fungal ligninolytic enzymes is promoted,

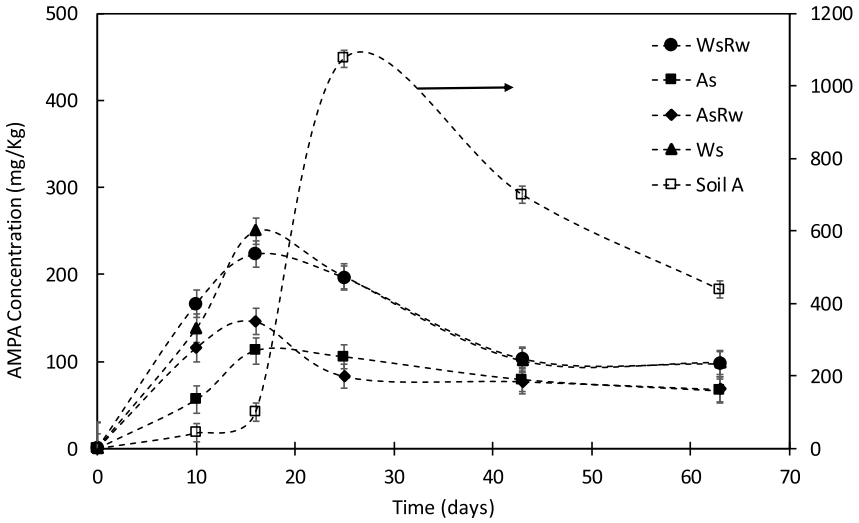

Fig. 3. AMPA concentration in biomixtures and soil. Each value is the mean of three replicates, and the errors bars show the standard deviation of the mean.

which correlates with the degradation of pesticides (Castillo and Torstensson, 2007). Pizzul et al. (2009) found an equal stoichiometry between AMPA formed and glyphosate degraded by the ligninolytic enzymes manganese peroxidase and laccase. Therefore in the present study only AMPA was analyzed. Peaks of this metabolite were observed in Fig. 3 during the first half of the experiment in all biomixtures and it was further degraded to concentrations between 60 and 100 mg kg^{-1} (Table 5). The maximum formation of AMPA in the biomixtures occurs at 16 days being As and AsRw the biomixtures with the lower generation of AMPA. This condition is related to lower glyphosate degradation in the same biomixtures (see Figs. 2 and 3).

In the control with only soil, glyphosate was degraded 53%. AMPA levels of 1100 mg kg^{-1} were detected after 23 days and at the end of the experiment the concentration of AMPA decreased to 438 mg kg^{-1}. These are important results since AMPA is more persistent than glyphosate to the biological degradation in soils, since AMPA is strongly sorbed by soils through the phosphonate group and protected against further microbial degradation (Borggaard and Gimsing, 2008) and many works have been published on its accumulation in soil and water (Mamy et al., 2010; Souza et al., 2006). The AMPA molecule accumulates in the soil since its generation is faster than its degradation (Simonsen et al., 2008). The same behavior is observed in the biomixtures studied by Roffignac et al. (2008) and in the present study.

In our study, we observed that the biomixtures containing river waste lost less water i.e. presented a better capacity to maintain a constant moisture content (Table S1). The water content is important for the biological activity in the biomixture and should be high enough to promote microbial processes and solubilization of pesticides but still leave enough pore space for oxygen to support aerobic processes (Castillo et al., 2008). Regulating the moisture content in farm BPS may be a laborious task for the farmers and therefore it is useful to have a material with good moisture retention capacity.

3.2. Biological activity

The number of total viable mesophilic bacteria (around 10^7 CFU g biomixture $^{-1}$) and yeast and fungi (around 10^5 CFU g biomixture $^{-1}$) did not vary significantly during the experiment (Table S2). These values suggest that glyphosate application did not significantly modify bacterial or fungi and yeast community, although no information was obtained on the incidence of some species through their identification.

In order to reinforce the obtained results, the effect of “days” on CFU g^{-1} for both bacteria and fungi was checked through one way ANOVA test. Taking “days” as a factor, since P-values obtained for each biomixture were greater than 0.05, the factor “days” has no statistically significant effect on the response “CFU g^{-1}” at the 95.0% confidence level.

Fig. 4 shows the initial and accumulated fluorescein hydrolytic activity (FDA) after 25 and 63 days. The effect of “days” and “biomixtures” on FDA activity was checked through multifactorial ANOVA test. Taking “days” and “biomixtures” as factors, since P-values of each factor were less than 0.05, both factors have a statistically significant effect on the response “FDA activity” at the 95.0% confidence level. Then the averages for the factor “biomixtures” were compared by Duncan multiple range test at the 95.0% confidence level.

The results showed that there are two homogenous groups (AsRw-S) and (As-WsRw-Ws) statically different. The addition of other materials to the soil enhanced FDA activity with the exception of biomixture AsRw, which showed an activity similar to the soil.

Even though AsRw and soil showed similar FDA activity, transformation of glyphosate was much higher in AsRw (Fig. 2), which indicates that other enzymes not evaluated could be involved in glyphosate degradation. More comprehensive studies are needed to determine the specific metabolic processes that were involved in the degradation of glyphosate and AMPA in the tested biomixtures. For example, fungal phenoloxidases are related to the degradation of glyphosate (Pizzul et al., 2009) and also lyases from bacterial origin may be present and can metabolize phosphonates through the rupture of the C—P bond, obtaining sarcosine as the intermediate in glyphosate degradation (Bozzo de Brum, 2010; Obojska et al., 1999), a metabolite that was not analyzed in this work.

4. Conclusions

All biomixtures showed higher glyphosate degradation capacity and less AMPA accumulation compared to the soil alone, being Ws the biomixture that present the highest glyphosate degradation rate. In addition, river waste is an alternative material to replace peat in biobed since it did enhance the water retention capacity. It can be concluded that alfalfa straw, wheat stubble and river waste are local materials that can be used in the preparation of biomixtures for pesticide degradation in BPS.

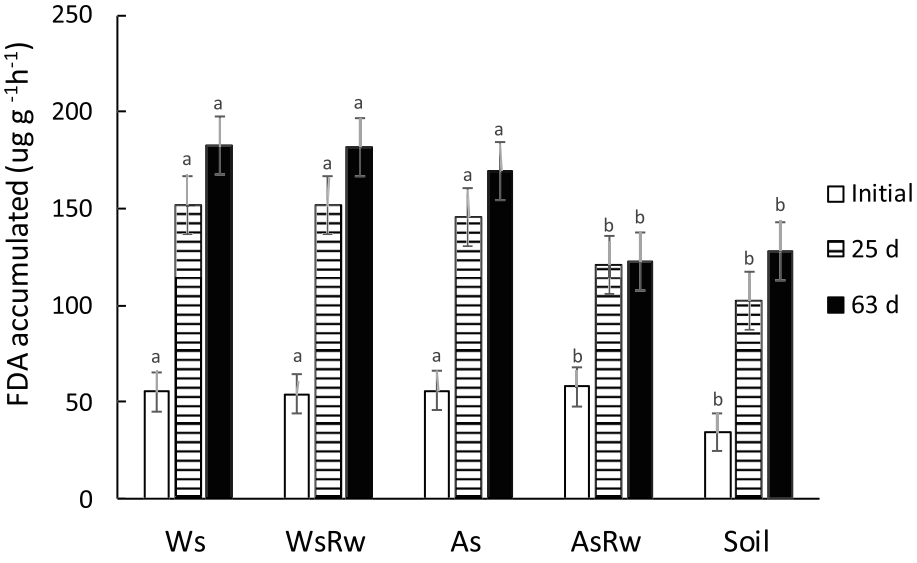

Fig. 4. Initial and accumulated FDA after 25 and 63 days for biomixtures and soil. Each value is the mean of three replicates, and the errors bars show the standard deviation of the mean. Different letters refer to significant differences between FDA accumulated means taking into account the factor "biomixtures" with Duncan test ($p < 0.05$).

Acknowledgment

We thank Ing. Matías Caillat from the Cooperativa Mixta Ltda (Margarita, Santa Fe, Argentina) for providing the materials used in this work and Daniela Buschiazzo, Melisa Mariani and Andrea Coll for their collaboration in part of the experimental work. This work was financed by CONICET, ANPCyT, SECTeI and UNL.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.09.009.

References

Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. J. Am. Water Resour. Assoc. 50, 275–290. https://doi.org/10.1111/jawr.12159.

Binimelis, R., Pengue, W., Monterroso, 2009. Transgenic treadmill: responses to the emergence and spread of glyphosate- resistant johnsongrass in Argentina. Geoforum 40, 623–633.

Borggaard, O., Gimsing, A., 2008. Review: fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag. Sci. 64, 441–456.

Bórtoli, P., Verdeneli, R., Conforto, C., Vargas, S., Meriles, J., 2012. Efectos del herbicida glifosato sobre la estructura y el funcionamiento de comunidades microbianas de dos suelos de plantaciones de olivo. Ecol. Austral 22, 33–42.

Bozzo de Brum, M.A., 2010. Tesis de Magíster en Ciencias Ambientales: Persistencia del glifosato y efecto de sucesivas aplicaciones en el cultivo de soja en agricultura continúa en siembra directa sobre parámetros biológicos del suelo. Universidad de la República, Facultad de Ciencias, Montevideo, Uruguay. http://ambiente.fcien.edu.uy/tesis/Tesis_Alexandra_Bozzo.pdf.

Casafe: Cámara de Sanidad Agropecuaria y Fertilizantes, 2013. La Argentina 2050. La revolución tecnológica del agro. Hacia el desarrollo integral de nuestra sociedad. Buenos Aires. Disponible en: http://www.casafe.org.

Castillo, M. d. P., Torstensson, L., Stenström, J., 2008. Biobeds for environmental protection from pesticide use. A review. J. Agric. Food Chem. 56, 6206–6219.

Castillo, M. d. P., Torstensson, L., 2007. Effect of biobed composition, moisture, and temperature on the degradation of pesticides. J. Agric. Food Chem. 55, 5725–5733.

Castro Berman, M., Marino, D., Quiroga, M.V., Zagarese, H., 2018. Occurrence and levels of glyphosate and AMPA in shallow lakes from the Pampean and Patagonian regions of Argentina. Chemosphere 200, 513–522.

Cuhra, M., Bøhn, T., Cuhra, P., 2016. Glyphosate: too much of a good thing? Front. Environ. Sci. 4, 1–14. https://doi.org/10.3389/fenvs.2016.00028.

De Wilde, T., Spanoghe, P., Debaer, C., Ryckeboer, J., Springael, D., Jaeken, P., 2007. Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. Pest Manag. Sci. 63, 111–128.

Di Benedetto, A., Plasman, R., Boschi, C., 2004. Use of river waste in growing media for ornamental herbaceous perennials. J. Hortic. Sci. Biotechnol. 79, 119–124.

Dick, R.E., Quinn, J.P., 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43, 545–550.

FAOSTAT 2016. http://faostat3.fao.org (most recent access date May 28 2018).

Gao, W., Liang, J., Pizzul, L., Zhang, K., Castillo, M.P., 2015. Evaluation of spent mushroom substrate as substitute of peat in Chinese biobeds. Int. Biodeterior. Biodegrad. 98, 107–112.

Góngora-Echeverría, V., Martin-Laurent, F., Quintal-Franco, C., Giácoman-Vallejos, G., Ponce-Caballero, C., 2017. Agricultural effluent treatment in biobed systems using novel substrates from southeastern Mexico: the relationship with physicochemical parameters of biomixtures. Environ. Sci. Pollut. Res. 24 (10), 9741–9753.

Grandcoin, A., Piel, S., Baurés, E., 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: its sources, behavior and environmental fate. Water Res. 117, 187–197.

Karanasios, E., Tsiropoulos, N., Karpouzas, D., 2012. On-farm biopurification systems for the depuration of pesticide wastewaters: recent biotechnological advances and future perspectives. Biodegradation 23, 787–802.

Karanasios, E., Tsiropoulos, N.G., Karpouzas, D.G., Menkissoglu-Spiroudi, U., 2010. Novel biomixtures based on local Mediterranean lignocellulosic materials: evaluation for use in biobed systems. Chemosphere 80, 914–921.

Karas, P., Metsoviti, A., Zisis, V., Ehaliotis, C., Omirou, M., Papadopoulou, E., Menkissoglou-Spiroudi, U., Manta, S., Komiotis, D., Karpouzas, D., 2015. Dissipation, metabolism and sorption of pesticides used in fruit-packaging plants: towards an optimized depuration of their pesticide-contaminated agro-industrial effluents. Sci. Total Environ. 530–531, 129–139.

Kawai, S., Uno, B., 1991. Determination of glyphosate and its major metabolite aminomethylphosphonic acid by high-performance liquid chromatography after derivatization with p-toluensulphonyl chloride. J. Chromatogr. 540, 411–415.

Lawrence, K.S., 2002. In: Vencill, W.K. (Ed.), Herbicide Handbook, eighth ed. Weed Science Society of America, pp. 231–234.

Li, H., Joshi, S.R., Jaisi, D., 2016. Degradation and isotope source tracking of glyphosate and aminomethylphosphonic acid. J. Agric. Food Chem. 64 (3), 529–538.

Mamy, L., Benoît, G., Barriuso, E., 2010. Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops. Environ. Pollut. https://doi.org/10.1016/j.envpol.2010.06.036.

Milan, M., Dalla Rovere, G., Smits, M., Ferraresso, S., Pastore, P., Marin, M.G., Bogialli, S., Patarnello, T., Bargelloni, L., Matozzo, V., 2018. Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel Mytilus galloprovincialis. Environ. Pollut. 237, 442–451.

Obojska, A., Lejczak, B., Kubrak, M., 1999. Degradation of phosphonates by streptomycetes isolates. Appl. Microbiol. Biotechnol. 51, 872–876.

Omirou, M., Dalias, P., Costa, C., Papastephanou, C., Dados, A., Ehaliotis, C., Karpouzas, D.G., 2012. Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production chain: laboratory, column and field studies. Environ. Pollut. 166, 31–39.

Pizzul, L., Castillo, M., Stenström, J., 2009. Degradation of glyphosate and other pesticides by ligninolytic enzymes. Biodegradation 20, 751–759.

Ratcliff, A., Busse, M., Shestak, C., 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl. Soil Ecol. 34, 114–124.

Roffignac, L., Cattan, P., Mailloux, J., Herzog, D., Le Bellec, F., 2008. Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalothrin under tropical climate conditions. Pest Manag. Sci. 64, 1303–1313.

Sasal, M.C., Demonte, L., Cislaghi, A., Gabioud, E.A., Oszust, J.D., Wilson, M.G., Michlig, N., Beldoménico, H.R., Repetti, M.R., 2015. Glyphosate loss by runoff and its relationship with phosphorus fertilization. J. Agric. Food Chem. 63 (18), 4444–4448. https://doi.org/10.1021/jf505533r.

Simonsen, L., Fomsgaard, I., Svensmark, B., Spliid, N.H., 2008. Fate and availability of glyphosate and AMPA in agricultural soil. J. Environ. Sci. Health B 43, 365–375.

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 6, 256–1261.

Souza, T.A., Matta, M.H.R., Montagner, E., Abreu, A.B.G., 2006. Estudo de recuperaçao de glifosato e AMPA derivados em solo usando resinas nacionais. Quim. Nova 29,

1372–1376.
Torstensson, L., Castillo, M. d. P., 1997. Use of biobeds in Sweden to minimize environmental spillages from agricultural spraying equipment. Pestic. Outlook 8, 24–27.
Tortella, G., Rubilar, O., Castillo, M.dP., Cea, M., Mella-Herrera, R., Diez, M.C., 2012. Chlorpyrifos degradation in a biomixture of biobed at different maturity stages. Chemosphere 88, 224–228.
Urrutia, C., Rubilar, O., Tortella, G.R., Diez, M.C., 2013. Degradation of pesticide mixture on modified matrix of a biopurification system with alternatives lignocellulosic wastes. Chemosphere 92, 1361–1366.
Wang, S., Seiwert, B., Kästner, M., Miltner, A., Schäffer, A., Reemtsma, T., Yang, Q., Nowak, K.M., 2016. (Bio) degradation of glyphosate in water-sediment microcosms - a stable isotope co-labeling approach. Water Res. 99, 91–100.
World Health Organization, 2015. Carcinogenicity of Tetrachlorvinphos, Parathion, Malathion, Diazinon, and Glyphosate. International Agency for Research of Cancer. www.thelancet.com/oncology.